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Agricultural Research Administration
Bureau of Entomology and Plant QuarantineLABORATORY TESTS WITH NEW COMPOUNDS AS INSECTICIDES AGAINST THE
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In an attempt to find a more effective insecticide for the pea aphid (Macrosiphum pisi (Kltb.)), tests have been conducted under greenhouse or laboratory conditions with a number of new organic materials. ^{1/} With the exception of DDT, most of the materials listed were not sufficiently toxic to be very promising.

The tests were made at Madison, Wis., by J. E. Dudley, Jr., and T. E. Bronson, in cooperation with the Wisconsin Agricultural Experiment Station, and at Columbus, Ohio, by F. H. Harries, in informal cooperation with the Ohio State University, during the period March 1941 to December 1943. For convenience, the results at the two locations will be presented separately.

Experiments at Madison, Wis.

Pea aphids were reared in the greenhouse on the English Broad Windsor bean (Vicia faba (L.)). After reaching maturity, the aphids were transferred for testing to pea plants grown in soil in No. 2 tin cans, each can containing one plant. When from 4 to 6 inches high each pea plant was infested with 10 or 15 newly transformed adult apterous aphids and immediately caged with a cylindrical screen-wire cage. Several hours later when the aphids had become settled on the plants, the cages were carefully removed, and all the plants for a treatment (1 to 6) were placed at the bottom of a glass cylinder 3 feet tall and 10 inches in diameter for dusting.

Dust mixtures were prepared in lots of 100 to 200 grams in a high-speed electric drink mixer, the type which has the revolving blades mounted in its base. A dosage of 100 to 500 mg. of dust mixture was expelled into the top of the cylinder through a copper dusting nozzle at an air pressure of 15 pounds per square inch. The top of the cylinder was at once covered and the dust allowed to settle for various lengths of time, after which the plants were removed, the wire cages immediately replaced over the plants, and the plants returned to the greenhouse benches for observation.

In the 1941 tests each of 13 synthetic organic compounds was diluted with an equal quantity of talc and tested at a dosage of 200 mg. of dust mixture against 10 aphids. Two tests were conducted, and the aphid mortality was recorded after 1, 2, and 3 days. The temperature averaged 64° F. during the first test and 71° during the second test. The following materials did not give appreciable mortality within 3 days after treatment:

^{1/} Most of the chemicals tested were furnished by the Division of Insecticide Investigations.

Diphenylamine, phthalonitrile, alpha, beta-dibromoethylbenzene (styrene dibromide), dibenzofuran, p-chloronitrobenzene, acetone semicarbazone, p-dibromobenzene, p-dichlorobenzene, and l-mercaptobenzothiazole.

Dibenzothiophene and 2,5-dichloroaniline killed most of the aphids at each temperature. 9,9-Dimethylacridan and 2-chloro-6-nitrotoluene were ineffective at 64° F. but within 3 days gave mortalities of 100 and 80 percent, respectively, at 71°. Foliage injury was caused by phthalonitrile, 2,5-dichloroaniline, 2-chloro-6-nitrotoluene, dibenzothiophene, and dibenzofuran.

Each of the materials that gave appreciable mortality at either temperature was tested at approximately 64° F. in one or more mixtures with derris. The results, as summarized in table 1, show that the effectiveness of derris was increased by the addition of the new materials. Incidentally, however, when mixtures containing 0.50 percent of rotenone, with and without 10 percent of 2,5-dichloroaniline, were applied to unreplicated field plots at the rate of 40 pounds per acre, both dust mixtures gave the same results.

Table 1.--Mortality of pea aphids dusted with 200 mg. of mixtures of synthetic organic materials and derris. Madison, Wis., 1941

Synthetic organic material	: Rotenone : : content : Aphids : : of mixture :		: Mortality after-- : 1 day : 2 days : 3 days			
	Percent	Percent	Number	Percent	Percent	Percent
2,5-Dichloroaniline	25	0.25	60	90	100	100
	10	.25	90	75	86	96
	10	.50	70	67	77	83
Dibenzothiophene	25	.25	20	100 ^{1/}	100	100
	10	.25	70	83	91	94
2-Chloro-6-nitrotoluene	25	.25	20	40	85	90
9,9-Dimethylacridan	25	.25	20	40	90	95
None (derris alone)	--	.50	100	40	48	64
None (check)	--	--	100	0	0	0

^{1/} Moderate foliage injury.

Undiluted ground thundergod vine (Tripterygium wilfordii (Hook. f.)) caused no mortality of the 20 pea aphids in two laboratory tests conducted at average temperatures of 64° and 72° F. A mixture containing 50 percent of thundergod vine and 2 percent of peanut oil in pyrophyllite was also ineffective.

During 1943 tests were made of a large number of specially prepared dust mixtures that had been sent to the laboratory by E. G. Beinhart, of the Eastern Regional Research Laboratory of the Bureau of Agricultural and Industrial Chemistry. Each mixture contained, in addition to other materials, 2 percent of nicotine derived from nicotine alkaloid, nicotine sulfate, or ground tobacco dust impregnated with nicotine alkaloid to contain 10 percent of nicotine. Pyrophyllite was used as the diluent. In each test 30 aphids were given a dosage of 250 mg. of the mixture.

The aphid mortality after 24 hours was 100 percent when the nicotine-pyrophyllite mixture contained 0.6 percent of a fractionated naphthalene oil. Lower mortalities were obtained when the same percentage of a refined oil, a lubricating oil (S.A.E. 10), a light and a heavy spray oil, or propylene laurate was substituted for the fractionated naphthalene oil. When the mixture contained 10 percent of sulfur and 1 percent of glycerol (C.P.), all the aphids were killed in 24 hours. The mixture became less effective when either the sulfur or the glycerol or both materials were omitted, and when either apple sirup (60 percent levulose) or a sulfonated oxidized petroleum hydrocarbon was substituted for the glycerol. The aphid mortality was 100 percent in 24 hours when the mixture contained 4 percent of a thiocyanate preparation (containing 50 percent of beta-thiocyanoethyl ester of an aliphatic acid and 50 percent of oil) but was less when smaller percentages of the thiocyanate were used.

In one series of tests with 30 aphids in each, dust mixtures containing talc impregnated with rotenone-containing resins dissolved in oil to contain 0.15 and 0.25 percent of rotenone were about as effective as comparable mixtures containing these percentages of rotenone in addition to 0.75 percent of phenoxathiin (phenothioxin). In similar tests a dust mixture containing 10 percent of talc and 90 percent of di(trimethylcyclohexenone) had no effect on the aphids. Very low aphid mortalities were obtained with various dust mixtures prepared from sabadilla seed furnished by T. C. Allen, of the University of Wisconsin. The powdered sabadilla seed was diluted with pyrophyllite and with talc to contain 33 and 60 percent of sabadilla. Other dust mixtures tested contained 5 and 10 percent of sabadilla seed extract in pyrophyllite. Low mortalities were also obtained when small percentages of rotenone were included in some of these sabadilla mixtures.

Tests were made of ground yam bean (Pachyrhizus erosus (Urban)) ^{2/} with pyrophyllite as the diluent, 30 aphids being used in each test.

^{2/} Furnished by R. Hansberry, of Cornell University, who in a processed report, "Notes on experiments with the yam bean," has summarized results of tests by various organizations with this material on the pea aphid and other insects.

Mortalities of less than 25 percent were obtained with dusts containing 10 and 20 percent of yam bean at dosages of 200 to 400 mg. of the mixture. The 40 and 60 percent yam bean mixtures, each at dosages of 300 and 500 mg., gave mortalities ranging from 60 to 93 percent. The temperature during the tests averaged from 63° to 70° F.

Results of tests with mixtures of DDT and pyrophyllite are summarized in table 2. These tests substantiate those reported by Harries (2) in showing that DDT is toxic to the pea aphid at relatively low strengths. The mortalities were much higher at 63°-65° F. than at 51°-55°.

Table 2.--Mortality of pea aphids dusted with 250 mg. of mixtures of DDT and pyrophyllite. Madison, Wis., 1943

DDT in mixture	Average temperature during test	Aphids	Mortality after--		
			1 day	2 days	3 days
<u>Percent</u>	<u>°F.</u>	<u>Number</u>	<u>Percent</u>	<u>Percent</u>	<u>Percent</u>
10	63	135	100	100	100
5	63	105	96	100	100
	55	45	91	100	100
1	64	165	68	98	100
	53	105	34	61	83
0.5	64	195	61	91	95
	53	105	45	61	82
.51/	65	105	54	99	100
	51	60	0	29	66
Checks	51-64	330	0	1	6

1/ Mixture also contained 1 percent of nicotine derived from ground tobacco dust impregnated with nicotine alkaloid to contain 10 percent of nicotine.

A series of tests was conducted on the residual effect of DDT when dusted on pea plants. The plants were dusted with mixtures of DDT and pyrophyllite and held for various periods before aphids were introduced. In all tests the average temperature was 64° F. The new plant growth (except as detailed in table 4) was removed from the plants prior to exposure to aphids in order to keep them from feeding on the untreated new foliage. In each test 45 adult aphids were used.

The residues from 500 mg. of the 5-percent and 10-percent strengths of DDT dust applied 1, 3, 5, 11, 14, and 21 days before exposure to aphids gave 84 percent mortality of the aphids in 1 day and 100 percent mortality in 3 days. The mortality of comparable check lots of aphids was 22 percent after 3 days. In other tests, summarized in table 3, the 1-percent and the 0.5-percent strengths of DDT had greater residual effect than did dust mixtures containing 0.5 percent of rotenone.

When the foliage was washed with tap water 8 days after being dusted with 10 percent DDT-pyrophyllite, enough residue remained to be very toxic to the 60 aphids that were placed on the foliage 1 day later (table 4).

Table 3.--Effect of exposure of pea aphids to foliage previously dusted with DDT or ground derris root mixed with pyrophyllite. Madison, Wis., 1943

Material and strength	Quantity applied	Interval between dusting of foliage and exposure to aphids	Aphids used	Mortality in 3 days
<u>Percent by weight</u>	<u>Milligrams</u>	<u>Days</u>	<u>Number</u>	<u>Percent</u>
DDT:				
1	250	10	45	100
		20	30	94
0.5	250	7	45	69
		9	45	78
Rotenone 0.5	250	9	45	44
	500	1	45	51
		3	45	51
		5	45	29
Rotenone 0.5 plus mineral oil 2	500	1	45	91
		3	45	96
		5	45	9
None (check)	---	-	240	8

Table 4.--Removal of DDT from pea foliage by washing 8 days after dusting, as indicated by the mortality of pea aphids placed on the foliage 1 day after washing. Madison, Wis., 1943

Treatment of foliage	Mortality after--		
	1 day	2 days	3 days
	<u>Percent</u>	<u>Percent</u>	<u>Percent</u>
Washed and new growth removed	70	92	98
Washed	18	38	54
New growth removed	97	100	100
None	88	96	100
Undusted (check)	0	0	0

Experiment at Columbus, Ohio

The methods employed at Columbus were similar to those used at Madison. The chief differences were that each mixture was applied at a very low dosage to 10 adult aphids by use of a bell-jar duster designed by Harries (1), and that the tests were usually replicated (repeated on different days.) Pea plants for use in the tests were sprouted in trays of sand in the laboratory and were prepared by transplanting two plants into each can of sand and adding 50 ml. of nutrient solution. The plants were placed under a bank of fluorescent lights, operated 12 or 14 hours daily with an electric time switch, and were used when about 3 to 4 inches high. After treatment the infested pea plants were ringed with a barrier to obviate the necessity of a screen cage and to avoid the effect of a loss of light. The plants were randomized in position under the same light conditions at temperatures of 70° to 80° F. and 40 to 60 percent relative humidity during the observation periods of 3 or 4 days.

The results, as summarized in tables 5 and 6, demonstrated that most of the materials were inferior to derris, although many of them were tested at the 50-percent strength. The mortalities were generally low even with derris, owing to the low dosages employed. DDT, however, was very effective and appeared to be more effective than derris at comparable strengths. Promising results were obtained with 2 percent of beta,beta'-dithiocyanodimethyl ether, 10 percent of yam bean, and 50-percent strengths of 4,6-dinitro-o-cresyl acetate, 2-chlorofluorene, 4,6-dinitro-o-cresyl methyl ether, and phenazine. The only other mixtures giving mortalities over 30 percent were those containing m-bromoacetanilide, phenoxathiin, and 2'-hydroxy-2,4,4',7-pentamethylflavan.

Table 5.--Mortality of pea aphids and the number of progeny 3 days after dusting with synthetic organic materials. Columbus, Ohio, 1943

Material	Mortality of adults	Surviving progeny per plant
	Percent	Number
Mixtures with equal parts of pyrophyllite, dosage 70 mg., 10 aphids in each of 7 replicates:		
Derris ^{1/}	43	18
m-Bromoacetanilide	33	40
Hydrazobenzene	20	24
Azobenzene	16	34
o-Iodonitrobenzene	16	31
m-Iodonitrobenzene	14	43
p-Iodoacetanilide	14	37
o-Iodoacetanilide	10	44
o-Chloroacetanilide	9	54
m-Chloroacetanilide	7	48
p-Bromoacetanilide	7	53
p-Chloroacetanilide	6	44
p-Iodonitrobenzene	6	48
None (check)	3	52
Mixtures with equal parts of talc, dosage 100 mg., each tested with 10 aphids repeated 6 times the same day:		
4,6-Dinitro-o-cresyl acetate	98	0
2-Chlorofluorene	90	1
4,6-Dinitro-o-cresyl methyl ether	75	3
Phenazine	63	4
Derris ^{1/}	62	7
Phenoxathiin (phenothioxin)	55	10
Phenothiazine	45	16
None (check)	7	54
Mixtures with talc, ^{2/} dosage 100 mg., 15 replicates of 20 aphids each:		
Rotenone 0.25% plus phenoxathiin 0.75%	45	88
Rotenone 0.25%	40	94
Rotenone 0.15% plus phenoxathiin 0.75%	40	97
Rotenone 0.15%	33	110
Rotenone 0.5% (from ground cube)	34	86
None (check)	4	196

^{1/} Diluted to contain 1 percent of rotenone.

^{2/} The first four dusts also contained 2 percent of oil and were prepared by combining derris resins and oil, with or without phenoxathiin, in acetone and spraying the mixtures into talc with thorough mixing.

Table 6.--Mortality of pea aphids and the number of progeny 3 days after dusting with various materials in comparison with rotenone; 10 aphids for each material in each replicate. Columbus, Ohio, 1943

Material	Mortality of adults	Surviving progeny per plant
	Percent	Number
Series A: Dosage 100 mg., 5 replicates		
DDT 10%:		
In pyrophyllite	100	0
Technical DDT diluted with proprietary 3% DDT dust	100	0
Rotenone 0.5% in derris-pyrophyllite	94	9
Yam bean 10% in diatomaceous earth-pyrophyllite	62	17
None (check)	4	59
Series B: Dosage 50 mg., 10 replicates		
Beta,beta'-dithiocyanodiethyl ether 2% in talc-magnesium carbonate:		
Lot 1	98	1
Lot 2	71	25
Rotenone in derris-pyrophyllite:		
0.3% from <u>Derris malaccensis</u>	40	65
0.5% from <u>Derris elliptica</u>	38	62
2'-Hydroxy-2,4,4',7-pentamethylflavan 5% in diatomaceous earth:		
Lot 1	31	70
Lot 2	34	67
None (check)	2	137
Series C: Dosage 100 mg., 5 replicates		
Rotenone 1% in derris-pyrophyllite	74	13
Piperine in pyrophyllite:		
16%	10	54
8%	6	55
4%	14	72
2%	22	53
1%	4	72
None (check)	0	96

Table 6.--(Continued)

Material	Mortality of adults	Surviving progeny per plant
Series D: Dosage 100 mg., 7 replicates		
Rotenone 0.5% in derris-pyrophyllite	46	21
Black pepper, undiluted	9	58
Black pepper in pyrophyllite:		
80%	11	49
40%	16	42
20%	7	56
10%	17	37
None (check)	3	80
Series E: Dosage 50 mg. ^{1/}		
DDT in pyrophyllite:		
10%	100	0
5%	100	0+
2.5%	98	2
1.25%	100	2
.625%	100	2
Rotenone 1% in derris-pyrophyllite	96	5
None (check)	21	108
Series F: Dosage 50 mg., 10 replicates ^{2/}		
DDT in pyrophyllite:		
10%	100	0+
5%	100	0
2.5%	99	3
1.25%	99	3
.625%	100	4
.325%	93	9
.156%	92	16
.078%	89	21
Rotenone 0.5% in derris-pyrophyllite	83	16
None (check)	3	123

^{1/} 80 aphids per treatment.

^{2/} Results with 9 of these replicates have been reported (Harries 2).



Literature Cited

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